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(U) THERMOPLASMA CYTOSKELETON AND CALMODULIN

12 PERSONAL AUTHOR(S)
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19 ABSTRACT (Continue on reverse if necessary and identify by block number)

(1) Electron micrographs have been obtained showing Thermoplasma acidophilum in amoeboid shapes attached to the surface of sulfur crystals. (2) Anti-actin antibodies reacted weakly with a 90 kDa protein in Western Blot analysis. Other cytoskeleton-related antibodies did not react. (3) Cytoplasmic extracts of T. acidophilum increased in viscosity upon incubation. Optimal conditions were approximately physiological. During incubation a subset of previously-soluble proteins became sedimentable by low speed centrifugation. (4) Extraction with non-ionic detergents left an insoluble residue of several protein species. (5) A calcium-affected protein has been identified as ferredoxin. It binds Ca^{++} with an affinity near 10^6 M^{-1}. (6) By autoradiography using phosphorus-32, hundreds of proteins are phosphorylated in vivo in T. acidophilum.

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DATE: May 25, 1989

ANNUAL REPORT: Contract N00014-88-K-0327

PRINCIPAL INVESTIGATOR: Dennis G. Searcy, Zoology Dept., University of Massachusetts, Amherst.

TITLE: Thermoplasma cytoskeleton and calmodulin.

PERIOD COVERED: 1 June 1988 to 31 May 1989 (year one).

OBJECTIVES: The primary goals are to examine the putative cytoskeleton of the Archaebacterium Thermoplasma acidophilum, regulation by intracellular calcium, and protein phosphorylation. Secondarily, characterization of the superoxide dismutase has continued.

PROGRESS (YEAR 1):

Cytoskeleton. Our observations confirm that a cytoskeleton is present. We have obtained scanning electron micrographs showing amoeboid cells attached to sulfur. (Sulfur is a respiratory substrate for T. acidophilum.) In the cold the cells round up suggesting that shape is maintained by some sort of temperature-labile activity.

Putative cytoskeletal and related proteins were examined using the Western Blot Technique. Antibodies tested included: anti-mouse myosin, anti-chicken gizzard intermediate filaments, anti-beef brain tubulin, anti-chick muscle actin, anti-chick embryo tubulin, anti-Giardia cytoskeletal proteins, and anti-cockroach calmodulin. All of these blots were negative except for the anti-actin, which weakly cross-reacted with a 90 kDa protein. (True actin is 42 kDa.) We interpret these results to be negative for proteins homologous to those of eukaryotes.

The tendency of the cytoplasmic extracts to increase in viscosity was examined. Extracts were made at 0°C using a low-salt extraction buffer that included sucrose, Mg-ATP, and BAPTA (a calcium chelator). Upon incubation at 20°C the extracts increased in viscosity. Quantitative data were obtained using a "rolling ball" viscometer. The data show that most of the viscosity increase occurred within 30 min. Experiments that

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methodically varied incubation conditions indicated that optimal viscosity increases occurred at 50 mM KCl, pH 6.5, free [Ca^{++}] > 10^{-7} M, and 35°C. Although 35°C is less than physiological (59°C), at higher temperatures a precipitate formed that apparently prevented viscosity development.

The experiments above were performed using a 10-fold dilution of the initial cell volume, and resulted in a 25% increase in viscosity. With higher concentrations of extract viscosity increases of up to 800% were obtained. At a lower pH gelation was observed.

DNase I did not interfere with the viscosity, showing that it was not caused by DNA. Nonetheless, in eukaryotes DNase I binds G-actin and should prevent cytoskeletal polymerization. Thus, the situation is different from that in eukaryotic cells. We interpret the viscosity increases described above to be evidence of filament polymerization or crosslinking, reflecting the activity of cytoskeletal elements.

Identification of the cytoskeletal proteins. During the viscosity increase a specific subset of proteins became sedimentable by low speed centrifugation (1000g). Extractions of live cells with the non-ionic detergents Triton X-100 or NP-40 have shown that a similar set of proteins are detergent-insoluble (but not an identical set: preliminary data).

Calcium-affected protein. Previously I had described an abundant protein in T. acidophilum that binds Ca^{++} in the presence of SDS resulting in a 20% increase in electrophoretic mobility. I had conjectured that the protein might be calmodulin. The protein has now been purified to homogeneity and is a ferredoxin. It binds calcium tightly, undergoing a conformational change that alters its ultraviolet absorbance properties, and increases its rate of electrophoretic migration in SDS. In contrast, control ferredoxins from spinach and from Sulfolobus acidocaldarius were not affected by calcium according to the electrophoretic mobility assay.

The amino acid sequence of the T. acidophilum ferredoxin is already known. There are no recognizable "E-F Hand" sequences, nor other similarities with eukaryotic calmodulin.

When assayed by the ultraviolet absorbance change, the binding affinity of the protein for Ca^{++} was estimated to be near 10^6 M^{-1}. Thus, there is the interesting possibility that Ca^{++} might be used to regulate the redox potential of the ferredoxin, or perhaps its kinetic rate constants. This is currently being examined.

Protein phosphorylation. This is an evolutionary marker that separates Eukaryotes from Eubacteria; phosphorylation occurs in eubacteria but affects only about 1% of the proteins. In contrast, in eukaryotic cells nearly all of the proteins are phosphorylated. When T. acidophilum cultures were grown for several generations in phosphorus-32 and then analyzed by 2-dimensional gel electrophoresis, hundreds of proteins were phosphate-labeled (but some proteins were not). This phenomonon is under further examination (and quantification).

Superoxide dismutase. We have previously described the T. acidophilum superoxide dismutase (SOD), which contains 2 Fe atoms and 1 Zn atom per homotetramer. Currently we are examining the environment of the metal atoms, which is presumed to be the active site of the enzyme. EXAFS spectra have been obtained in collaboration with Dr. Michael Maroney, Chemistry Dept., Univ. of Mass., and Mossbauer spectra have been obtained in collaboration with Dr. Peter Debrunner, Univ. of Illinois. Preliminary data suggest that the active site likely includes both iron and zinc. If confirmed, this will be a new type of SOD, since in other known SODs the active site might include 2 iron atoms, but not zinc. Efforts are now under way to prepare a zinc-free protein which should resolve certain ambiguities in the EXAFS data.

The partial amino acid sequence of the protein has been obtained by Dr. Brian Edwards, Wayne State University. It is 32% identical with Halobacterium cutirubrum SOD. This is less similarity than exists between H. cutirubrum and E. coli SODs (39%). Thus, although the SOD is homologous to those of other bacteria, it is only very distantly so, consistent with the suggestion above that it might be a new type of SOD.